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Short Communication

Validation of growth zone deposition rate in otoliths and scales of flathead mullet *Mugil cephalus* and freshwater mullet *Myxus capensis* from fish of known age

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Flathead mullet *Mugil cephalus* and freshwater mullet *Myxus capensis* are important components in South African estuarine fish communities and fisheries, but there is little information on their age and growth or age validation. This study validated the periodicity of growth zone formation in sectioned sagittal otoliths and scales of fish of known age that had been at liberty for 10 years. For both *M. cephalus* and *M. capensis*, the number of growth zones counted on otolith sections did not differ significantly from the known age of the fish. There were significantly fewer growth zones on scales than the known age of both *M. cephalus* and *M. capensis*. Growth zone deposition rate could therefore be validated as annual for otoliths but not for scales of the two species. It is recommended that future ageing studies focus on otoliths rather than scales.

Keywords: age validation, growth zones, sagittal otoliths, South Africa

Introduction

Globally, mullets are dominant components in many estuarine fish communities (Smith and Deguara 2003) and are important in many capture fisheries (Lamberth and Turpie 2003). Some mugilids have also been used as indicator species for environmental health (Whitfield and Elliot 2002, Whitfield et al. 2012). In South Africa, mullets occur in all estuaries where they collectively comprise more than 30% of the biomass (Harrison 2005). They are important in artisanal fisheries in the Orange River and Kosi lakes systems and are utilised by recreational and subsistence anglers and cast netters for food and bait (Kyle 1999, Lamberth and Turpie 2003).

Mugil cephalus (Linnaeus 1758) is a cosmopolitan species distributed widely in coastal and estuarine waters of the tropical, subtropical and temperate zones of all seas (Whitfield et al. 2012), whereas *Myxus capensis* (Valenciennes 1836) is endemic to southern Africa (Whitfield 1998). Both are large species that attain lengths in excess of 500 mm fork length (FL). They are able to tolerate a wide range of salinities (*M. cephalus*: 0–84; *M. capensis*: 0–49) and considerable portions of their populations make use of freshwater habitats, sometimes penetrating up to 100 km up rivers (Bok 1979, Chang et al. 2004). Increasingly, these migrations are being impacted by instream barriers that now limit the freshwater distribution of these fish (Wasserman et al. 2011) and by non-native fish that prey on migrating juveniles of the two species (Weyl and Lewis 2006). Understanding the biology of these mullet species is therefore important not only for understanding their role in fish communities but also for their management and conservation.

Accurately determining fish age is fundamental to understanding their biology (Campana 2001). In mullets, age estimates are generally obtained by counting growth zones on calcified structures such as scales (Hsu and Tzeng 2009) and otoliths (Smith and Deguara 2003). As is the case with all analyses of hard parts, the accuracy of the ages estimated from these counts requires validation, because the frequency of growth zone formation can vary not only between species but also between populations of the same species (Campana 2001, Winker et al. 2010). In addition, interpretation of growth zone deposition by different readers may introduce additional bias (Campana 2001). In his review of methods for validating growth zone deposition rates in calcified structures of fish, Campana (2001) concluded that the most rigorous method of validation was through correlating the number of growth zones on hard parts with the known age of fish.

Relatively few studies have validated growth zone deposition rates in *M. cephalus* and *M. capensis*. In *M. cephalus*, the periodicity of growth zone formation in hard parts has been validated by direct methods including the mark recapture of chemically marked wild caught captive fish in Australia and Japan (Chang et al. 2000, Smith and Deguara 2003) and from known age individuals in Japan (Hsu and Tzeng 2009). In South Africa, the only validation study on *M. capensis* and *M. cephalus* was undertaken using scales of fish of known age (Bok 1983). The aim of the present study was to validate growth zone deposition rate in both scales and otoliths using *M. capensis* and *M. cephalus* of known age.

Material and methods

Mugil cephalus and *M. capensis* were introduced into Binfield Park Dam as a single stocking of 75 000 wild caught, young-of-the-year (20–40 mm total length, TL) fish collected from the Keiskamma River estuary on 13 September 2000 (A Bok, Anton Bok Consultants, Port Elizabeth, pers. comm.). As neither species is capable of reproducing in fresh water (Bok 1979), these stocked fishes provided the opportunity to validate growth zone deposition rate from fish of known age.

Binfield Park Dam (32°41.459′ S, 26°54.585′ E) is a 260 ha impoundment situated on the Tyume River; a headwater tributary of the Keiskamma River system, Eastern Cape, South Africa (Figure 1). The impoundment is located 220 km inland at an altitude of 665 m above sea level, and the climate is warm-temperate with a mean ambient temperature of 18 °C and mean annual rainfall of approximately 400 mm (Schulze et al. 2008).

The fish population in the dam was sampled on 12–14 July 2011 using multifilament gillnet fleets. Sampled fish were measured to the nearest mm FL, weighed to the nearest 0.1 g, dissected and sexed. The sagittal otoliths, and scales from between the lateral line and the dorsal fin, were collected and stored dry for later examination.

Preparation of otoliths and scales

Otoliths were cleaned, set in clear polyester casting resin, sectioned transversely through the nucleus (0.3 mm using a double-bladed diamond edge saw), mounted on slides using DPX mountant, and viewed under a dissecting microscope using transmitted light (variable magnification:10–40×). Cleaned scales were placed between two transparent glass slides on a black background and read under a dissecting microscope using reflected light (magnification: 10×).

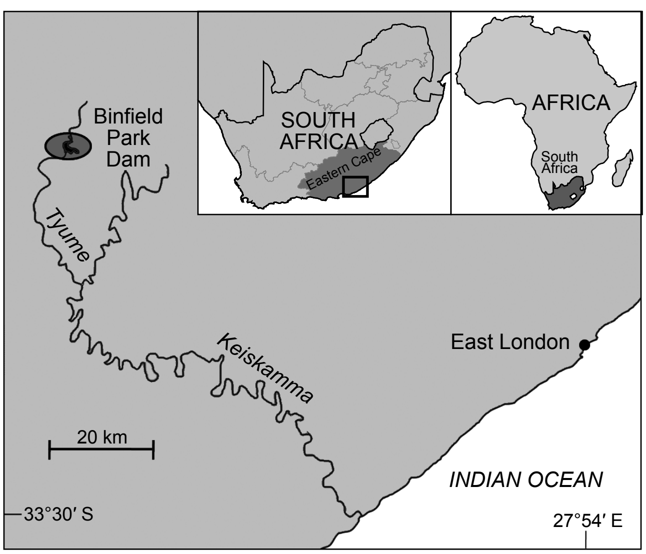

Figure 1: Location of Binfield Park Dam, situated on the Tyume River, a headwater tributary of the Keiskamma River, Eastern Cape, South Africa

In both structures, growth was reflected as a series of alternating opaque and hyaline bands (Figures 2, 3). According to common practice (Hsu and Tzeng 2009), one opaque and the successive hyaline band was considered to be a growth zone. Structures were read by three readers that had no prior knowledge of the size or expected age of the fish. The mode of these three readings was then adopted for each sample as the estimated age for each fish. If no mode could be calculated, or if one of the samples was considered to be unreadable by one of the readers, then all data from that sample were excluded from further analysis.

Analysis

To test the hypothesis that growth zone deposition was annual, a two one-sided *t*-test (TOST) was used to test for differences between the estimated age and the known age of the fish (Limentani et al. 2005). The limits (or θ) were set to 5%, which is equivalent to 0.5 years either side of the known age of 10 for *M. cephalus* and *M. capensis*.

To determine the most accurate structure for ageing, precision of the age estimate for each structure was estimated using the index of average percentage error (IAPE) (Beamish and Fournier 1981) and the coefficient of variation (CV) (Campana et al. 1995).

Results

Mugil cephalus

The sample of *M. cephalus* consisted of 13 females, two males and five unsexed individuals, ranging in length from 445 to 636 mm FL. Growth zone counts ranged from 5 to 9 for scales and 9 to 11 for otoliths (Table 1). Seven *M. cephalus* scales were rejected due to an inconsistency in the age estimates between readers. Readings were more precise for otoliths (IAPE = 5.24%, CV = 7.21%) than for scales (IAPE = 13.2%, CV = 19.2%). Scale readings differed

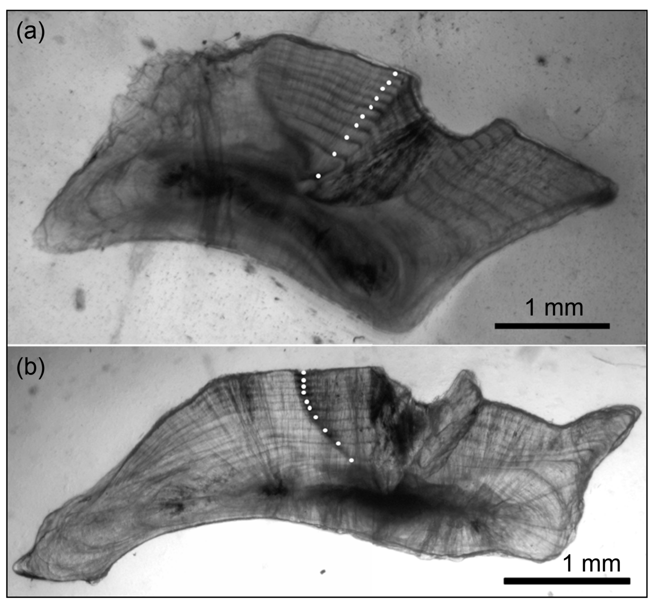

Figure 2: The sectioned sagittal otoliths illustrating annuli of (a) a female *Myxus capensis* of 536 mm FL and (b) a male *Mugil cephalus* of 414 mm FL recaptured from Binfield Park Dam

significantly from the known age of the fish (TOST, t = 1.71, df = 13, 11, difference = −29.2%). Otolith readings did not differ significantly from the known age of the fish (TOST, t = 1.68, df = 20, 18, difference = −1.5%), supporting the hypothesis that growth zone deposition rate is annual for *M. cephalus*.

Myxus capensis

The sample of *M. capensis* consisted of eight females and three males, ranging in length from 386 to 490 mm FL. Growth zone counts ranged from 5 to 10 for scales and 9 to 10 for otoliths (Table 1). Two otoliths and five scales were rejected due to inconsistencies between readers. Readings

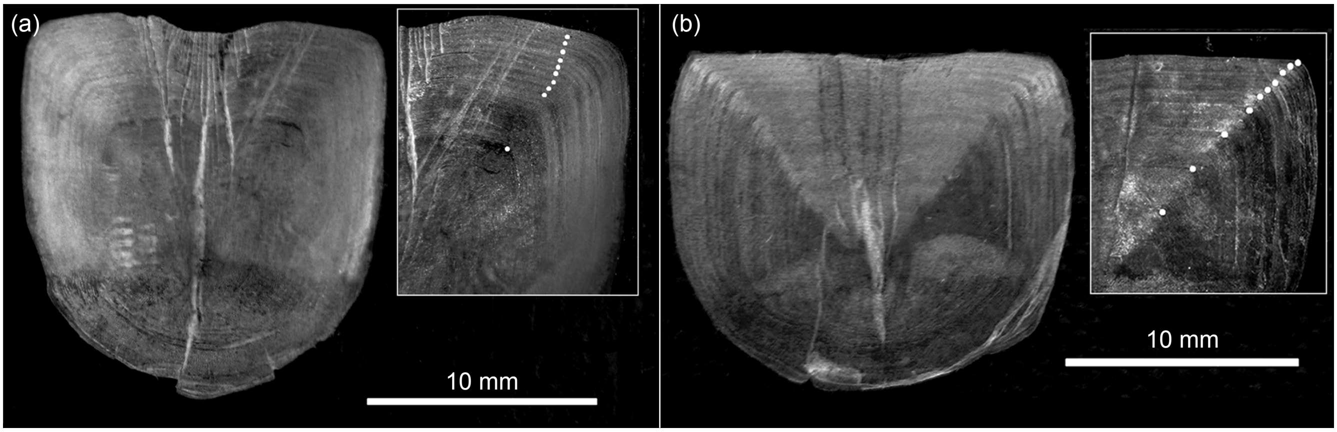

Figure 3: Scales used to illustrate the annuli of (a) a *Mugil cephalus* female fish of 510 mm FL and (b) a *Myxus capensis* female fish of 454 mm FL recaptured from Binfield Park Dam

Table 1: Summary of recaptured *Mugil cephalus* and *Myxus capensis,* stocked at 20–40 mm TL on the 13 September 2000 into Binfield Park Dam, Eastern Cape, South Africa. Number of annuli reflects the modal age from the three readings for otoliths and scales

Recapture date	Time at liberty (years)	Fork length (FL, mm)	Sex	Known age (years)	Number of annuli (otoliths)	Number of annuli (scales)
			M. cephalus			
12 July 2011	10.67	536	F	10	10	7
12 July 2011	10.67	525	F	10	10	9
12 July 2011	10.67	535	F	10	9	8
12 July 2011	10.67	475	M	10	9	9
12 July 2011	10.67	520	F	10	9	*
12 July 2011	10.67	560	F	10	10	*
12 July 2011	10.67	445	M	10	10	6
12 July 2011	10.67	506	F	10	10	7
12 July 2011	10.67	530	F	10	9	*
12 July 2011	10.67	636	F	10	10	7
13 July 2011	10.67		F	10	10	7
13 July 2011	10.67	507	F	10	10	*
13 July 2011	10.67	510	F	10	10	8
13 July 2011	10.67	499	F	10	10	*
13 July 2011	10.67	509	F	10	10	*
13 July 2011	10.67	510		10	11	*
13 July 2011	10.67	520		10	10	7
13 July 2011	10.67	550		10	10	5
13 July 2011	10.67	513		10	10	6
13 July 2011	10.67	505		10	10	6
12 July 2011	10.67	471	F	10	*	*
			M. capensis			
12 July 2011	10.67	395	M	10	9	*
12 July 2011	10.67	475	F	10	*	7
12 July 2011	10.67	475	F	10	10	7
12 July 2011	10.67	454	F	10	9	7
12 July 2011	10.67	456	F	10	9	*
12 July 2011	10.67	468	F	10	10	10
13 July 2011	10.67	490	F	10	10	*
13 July 2011	10.67	414	M	10	10	9
13 July 2011	10.67	465	F	10	10	*
13 July 2011	10.67	386	M	10	9	5

*** =** Data discarded from scales/otoliths due to inconsistencies between readings were more precise for otoliths (IAPE = 8.47%, CV = 11.43%) than for scales (IAPE = 19.0%, CV = 26.1%). Scale readings differed significantly from the known age of the fish (TOST, t = 1.81, df = 6,4, difference = −25.0%). Otolith readings did not differ significantly from the known age of the fish (TOST, t = 1.75, df = 9,7, difference = −4.5%), indicative that growth zone deposition rate is annual for *M. capensis*.

Discussion

Our results validate that growth zone deposition rate in *M. cephalus* and *M. capensis* otoliths is annual. However, growth zone counts from scales were often lower than the known age of the fish (Table 1). The annual growth zone deposition rate is consistent with results from validation experiments on members of the family Mugilidae elsewhere (Chang et al. 2000, Smith and Deguara 2003, Hsu and Tzeng 2009). Although the mullet used in this study were from a population introduced into a large freshwater impoundment, we consider the results of this study as valid because both *M. cephalus* and *M. capensis* spend considerable parts of their life history in freshwater environments (Bok 1979, Whitfield 1998, Chang et al. 2004).

Whereas scales have been considered to be reliable estimators of age for the two species under study (Bok 1983), the current study demonstrates that scales may underestimate age for older fish. This finding concurs with similar studies on other mullet species (Ibáñez-Aguirre and Gallardo-Cabello 1996, Smith and Deguara 2003, Hsu and Tzeng 2009). Thus, it is recommended that future ageing studies should focus on the use of sectioned sagittal otoliths. While we consider otoliths as the more appropriate structure for ageing these species, our results indicate that growth zone deposition rate was most likely annual in scales as well, particularly for young fish. Scales can be collected without having to sacrifice the fish, and because previous research on the two mullet species was conducted using scales, research determining at what age scale- and otolith-based age estimates begin to deviate would be valuable. It is, however, essential that direct validations of wild stocks through techniques such as the mark recapture of chemically tagged wild fish be conducted if long-term ageing studies are to be attempted.

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